Table K. Cumulative mg. CHS-Na excreted to Various Times following Oral Administration of 5.0 g. or 1.0 g. Dose in Humans

Dose (g.)	Subject[a]	mg. excreted, hr. 1	2	3	4	5	6	24
5.0	S.K.(33～50) ♂	132	267	365	454	580	775	3100
〃	S.H.(23～52) 〃	—	—	—	—	—	—	2390
〃	M.D.(22～60) 〃	—	—	—	—	—	—	1765
1.0	S.K.(33～50) 〃	22	50	73	91	—	154	382
〃	S.H.(23～52) 〃	—	—	—	—	—	—	420

a) Bracketed quantities are subject's age in years followed by his weight in kilograms.

Summary

Studies on the absorption and excretion of CHS-Na in rabbit and human are reported in this paper.

The urinary excretion rate of CHS-Na was determined after oral administration to rabbit, and the absorption rate was calculated from the excretion data.

And the absorption of CHS-Na was studied after oral administration with the compounds such as caffeine, theophylline, theobromine, albumin, casein, and citric acid, and it was suggested that the absorption of CHS-Na was accelerated in the presence of caffeine and citric acid.

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131. Shoji Kojima, Hisashi Ichibagase,[*1] and Sadao Iguchi[*2] : Studies on Synthetic Sweetening Agents. Ⅶ.[*3] Absorption and Excretion of Sodium Cyclamate. (2).

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In the previous report,[*3] the absorption and the excretion of sodium cyclamate (CHS-Na) were studied in rabbit and human.

The present report describes the absorption of CHS-Na from the stomach or the small intestine of rat *in situ*. And the absorption experiments of saccharin and dulcin were also carried out with the same method and were compared with that of CHS-Na.

In those experiments described above, the absorption of CHS-Na in rat *in situ* was shown to be less than *in vivo*. In order to investigate the causes of the reduction of the absorption, the effects of pentobarbital, which was used as anesthetic in those experiments, on the absorption of CHS-Na were also studied in rabbit and rat.

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*3 Part Ⅵ. S. Kojima, H. Ichibagase, S. Iguchi : This Bulletin, 14, 959 (1966).

Furthermore, the absorptions of CHS-Na from the stomach and the small intestine of rat were investigated in the presence of the some kinds of compounds, which were used in the previous report.*3

Experimental

Materials——CHS-Na was recrystallized from water, dried at 105° for 2 hr. Dulcin and saccharin were recrystallized from water, dried over anhyd. $CaCl_2$. Salicylamide was of J.P. Ⅶ grade. Tolazoline injection contanining 10 mg./ml. was used.

Animals——Male rats weighing 140 to 180 g. and male rabbits weighing 3 to 4 kg., maintaining on the solid food*4 and water, were fasted for about 20 hr. prior to the experiments. Water was given freely during the experiments and coprophagy was prevented by using the special cage.

Absorption Experiments in Stomach and Small Intestine of Rats——Absorption experiments were carried out according to the method of Schanker, *et al.*[1,2]

The small intestine was flashed with 50 to 100 ml. of 0.9% NaCl solution, maintained at 37°, and then with 30 ml. of sample solution. The tubings attached to the inflow and outflow polyethylene cannulae were then connected to a flask containing 30 ml. of sample solution as shown in Fig. 1. The sample solution was then continuously circulated at a rate of 3.0 ml. per minute through the small intestine for 2 hr. at 37°, using the circulated apparatus shown in Fig. 1. Four ml. of the sample solution was pipetted at 1 hr. after the circulation was started. The concentrations of the sample and indicator (phenol red) were determined. The absorption rate was calculated from the decrease in the sample concentration, and was corrected for the volume change by the equation (1).

$$\text{Per cent absorbed} = 100 - 100\left(\frac{C_{\text{sample final}}}{C_{\text{sample initial}}} \times \frac{C_{\text{phenol red initial}}}{C_{\text{phenol red final}}}\right) \qquad (1)$$

Each sample solution which contained 400 μg./ml. of CHS-Na, 360 μg./ml. of dulcin, or 2000 μg./ml. of saccharin was prepared by dissolving in the isotonic buffer solution as shown in Table I. Phenol red which was expected to be unabsorbed,[1,2] was dissolved in those sample solutions to indicate the volume change.

Table I. Isotonic Buffer Solutions used for Sample Solution

pH	*N* HCl (ml.)	10% Citric acid (ml.)	10% Na_2HPO_4 $12H_2O$ (ml.)	10% KH_2PO_4 (ml.)	2*N* NaOH (ml.)	0.1*M* Glycine (ml.)	10% NaCl (ml.)	H_2O
1.0	100.0	—	—	—	—	—	61.0	
2.9[a]	2.0	—	—	—	—	80.0	57.0	
3.0	62.0	84.0	—	—	40.0	—	34.0	to make
5.0[a]	—	—	4.8	89.0	—	—	50.0	1000 ml.
5.0	—	210.0	—	—	100.0	—	—	
7.0	—	—	143.0	36.0	—	—	43.0	

a) These buffer solutions were used for saccharin.

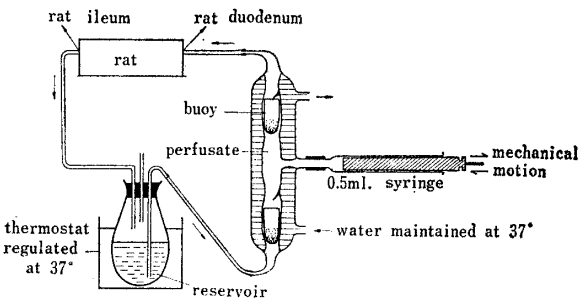

Fig. 1. Apparatus used for Circulation Experiments of Rat Small Intestine

Absorption Experiments *in vivo*——In rats : 30 mg. of CHS-Na dissolved in 1.5 ml. of water was administered orally to rats or the pentobarbital-anesthetized rats. And 1.3 mg. of tolazoline was administered similarly. The animals were kept in the cage and sacrificed at the desired time. The urine was collected by an injection-syringe from the bladder, which was exposed by a midline abdominal incision, and was combined with the urine excreted. The combined urines were diluted with water to 20~40 ml. and submitted to the determination of CHS-Na or tolazoline.

*4 "CR-1 and CE-2", Nippon Clea Co., Ltd. were used.

1) L.S. Schanker, P.A. Shore, B.B. Brodie, A.M. Hogben : J. Pharmacol. Exp. Therap., **120**, 528 (1957).
2) L.S. Schanker, D.J. Tocco, B.B. Brodie, A.M. Hogben : *Ibid.*, **123**, 81 (1958).

In rabbits : 600 mg. of CHS-Na in 20 ml. of water or 1 g. of salicylamide suspended in 20 ml. water was administered orally to rabbits and the pentobarbital*5-anesthetized rabbits. In the case of the latter, each 40 mg. of pentobarbital was administered by intravenous injection successively at 2.5, 4.5 and 6.5 hr. after administration. The urine was collected by Nelaton's catheter at 0~1, 1~2, 2~3, 3~4, 4~5, 5~6, and 6~8 hr., was diluted with water to 10~50 times, and then submitted to the determination of CHS-Na or tolazoline.

Determination of Partition Coefficients——Each sample solution containing 400 μg./ml. of CHS-Na, 360 μg./ml. of dulcin, or 2000 μg./ml. of saccharin was prepared by dissolving in buffer solution of pH 1.0, 5.0 and 7.0. To 5 ml. of sample solution the equal volume of organic solvent was added. These were kept in a water-bath at 37° with vigorous shaking every 5 minutes for 1 hr. Then the sample content was determined against water layer, and partition coefficients (K) were calculated by the equations (2) and (3).

$$K=\frac{\text{Initial concn. of water layer}-\text{Equild. concn. of water layer}}{\text{Equild. concn. of water layer}} \qquad (2)$$

$$K=\frac{\sqrt{\text{Initial concn. of water laver}-\text{Equild. concn. of water laver}}}{\text{Equild. concn. of water layer}} \qquad (3)$$

Dissociation Constant——The dissociation constants for cyclohexylsulfamic acid and saccharin were determined by the potentiometric titration in aqueous solution. Fifteen ml. of $10^{-1}\,M$ cyclohexylsulfamic acid solution and 50 ml. of $2\times10^{-2}\,M$ saccharin solution were titrated with $0.1\,M$ NaOH, and the pH of these solutions were measured with Horiba pH meter, Model-H. The dissociation constant for dulcin was determined by the spectrophotometric method using 0.1 to $4.0\,N$ HCl, and $0.025\,M$ Walpole acetate and $0.015\,M$ Sörensen phosphate buffers.

Analytical Methods——Sample solution containing CHS-Na was diluted with water to give a concentration of about 100 μg./ml. and analyzed by the method reported previously.[3] Dulcin was extracted with AcOEt from the sample solution which was made alkaline with $0.25\,N$ NaOH, and the extract was evaporated to dryness. The residual dulcin was dissolved in water and determined according to the method reported previously.[4] Saccharin was extracted with AcOEt from the sample solution which was acidified with $1N$ HCl, and the extract was evaporated to dryness. The residue was dissolved in warm water and determined by the titration method with $0.1\,N$ NaOH. Salicylamide was determined according to Kakemi, *et al.*[5] Tolazoline was determined according to Brodie, *et al.*[6] Sample solution containing phenol red was appropriately diluted with $1N$ NaOH. The optical density was measured at 550 mμ.

Results

Absorption of CHS-Na, Saccharin, and Dulcin from Rat Stomach

The gastric absorption of CHS-Na, saccharin, and dulcin during 1 hour is shown in Table II. CHS-Na was very poorly absorbed in the solution of pH 1.0, and was not

Table II. Absorption Rate of CHS-Na, Saccharin, and Dulcin from the Rat Stomach

Compound	pKa	Per cent absorbed in 1 hr.[a]	
		pH 1.0	pH 3.0
CHS-Na	1.9[b]	4.1±1.3(7)	0 ±0 (4)
Saccharin	2.2	16.6±0.9(3)	8.6±2.6(3)[c]
Dulcin	<0.2	24.5±3.5(3)	22.2±4.8(4)

a) The per cent absorbed is expressed as the mean ± the range followed by the number of experiments in parentheses.
b) The value shows pKa of cyclohexylsulfamic acid.
c) pH in initial sample solution was 2.9.

*5 40 mg. per kg. wt.

3) S. Kojima, H. Ichibagase : Yakugaku Zasshi, **83**, 1108 (1963).
4) H. Ichibagase, S. Kojima, M. Ichikawa : Yakugaku Zasshi, **84**, 707 (1964).
5) K. Kakemi, T. Arita, H. Yamashita, R. Konishi : Arch. Pract. Pharm., **21**, 103 (1961).
6) B. B. Brodie, L. Aronow, J. Axelrod : Pharmcol. Exp. Therap., **106**, 200 (1952).

absorbed in the solution of pH 3.0 The absorption rate of saccharin was more than that of CHS-Na in both solutions, and then the absorption in the solution of pH 3.0 was significantly less then in solution of pH 1.0. On the other hand, dulcin had the greatest absorption value among all in both solutions of 1.0 and 3.0.

Influence of gastric juice on the pH of sample solutions was scarcely recognized.

Table Ⅲ. Absorption Rate of CHS-Na, Saccharin, and Dulcin from the Rat Small Intestine

Compound	pKa	Per cent absorbed[a)]			
		pH 5.0		pH 7.0	
		1 hr.	2 hr.	1 hr.	2 hr.
CHS-Na	1.9	4.0±1.5(5)	8.1±1.0(3)	4.7±0.2(2)	8.0±0.7(5)
Saccharin	2.2	4.6±0.3(3)	9.5±2.3(3)	4.2±0.5(3)	7.0±1.5(4)
Dulcin	<0.2	69.8±1.4(3)	81.4±1.6(3)	69.8±4.8(3)	87.8±2.0(3)

a) The per cent absorbed is expressed as the mean ± the range followed by the number of experiments in parentheses.

Absorption of CHS-Na, Saccharin, and Dulcin from Rat Small Intestine

The small intestinal absorption of CHS-Na, saccharin, and dulcin was determined at 1 and 2 hours after the circulation was started. The results are shown in Table Ⅲ.

CHS-Na and saccharin were slowly absorbed (less than 9 per cent during 2 hours), while dulcin was rapidly absorbed (more than 80 per cent during the same period).

The extent of pH shift of the sample solutions during the circulation was 5.3 to 6.1 or 6.6 to 6.9 at initial sample solution of pH 5.0 or 7.0 respectively.

Partition Coefficients

Partition coefficients were measured in three kinds of sample solutions of pH 1.0, 5.0, and 7.0 using isoamyl acetate and chloroform as organic solvents. The partition coefficient of saccharin between isoamyl acetate and the sample solution of pH 1.0 was calculated by the equation (3). Other partition coefficients were obtained from the equation (2). Partition coefficients obtined are shown in Table Ⅳ.

Table Ⅳ. Partition Coefficients of CHS-Na, Saccharin, and Dulcin

Compound	Isoamyl acetate			Chloroform		
	pH 1.0	pH 5.0	pH 7.0	pH 1.0	pH 5.0	pH 7.0
CHS-Na	0.015	0.015	0.0	0.012	0.0	0.0
Saccharin	3.23	0.37	0.0	1.48	0.09	0.016
Dulcin	1.96	2.04	2.15	1.36	1.41	1.45

Influence of Anesthetic on Absorption of CHS-Na, Salicylamide, and Tolazoline in Rat and Rabbit *in vivo*

Influence of pentobarbital used as an anesthetic on absorption of CHS-Na, salicylamide, and tolazoline was studied in rat and rabbit *in vivo*. The difference of the absorption in normal and anesthetized animals was examined by determining those excretion amounts after oral administration.

The absorption of CHS-Na was suppressed with pentobarbital in rat and rabbit, and the absorption of tolazoline was also suppressed in rat. But the effect of pentobarbital on the absorption of salicylamide was scarcely recognized in rabbit. The results obtained are shown in Table V.

Table V. Influence of Pentobarbital on Absorption of CHS-Na, Salicylamide, and Tolazoline in Rat or Rabbit

Compound	Animal	Pento-barbital	Oral dose (mg.)	Exp. No.	Per cent excreted, hr.[a]						
					1	2	3	4	5	6	8
CHS-Na	Rat	—	30	3	—	—	—	—	23.2	—	—
	〃	+	30	2	—	—	—	—	12.5	—	—
	Rabbit	—	600	4	2.4	11.1	22.7	34.7	45.5	54.6	67.9
	〃	+	600	3	0.3	0.7	1.8	4.7	8.0	12.0	26.0
Salicylamide	Rabbit	—	1000	2	—	26.5	—	46.1	—	53.4	59.4
	〃	+	1000	2	—	18.0	—	45.6	—	54.6	59.9
Tolazoline	Rat	—	1.3	3	13.8	—	—	—	80.8	—	—
	〃	+	1.3	3	4.7	—	—	—	63.5	—	—

a) The per cent excreted is expressed as the mean value.

Effect of Other Compounds on Absorption of CHS-Na from Stomach and Small Intestine of Rat *in situ*

Other compounds such as caffeine, theophylline, theobromine, albumin, casein, and citric acid were used to study the effects on the absorption of CHS-Na from stomach and small intestine. And the absorption rates of CHS-Na in isotonic buffer solutions,

Table VI. Absorption Rate of CHS-Na from Rat Stomach in the Presence of Other Compounds using Isotonic Buffer Solution of pH 1.0

	Concn. in sample soln. (μg./ml.)	Per cent absorbed in 1 hr.[a]
Caffeine	400	3.8±1.0(4)
Theophylline	400	3.6±0.6(2)
Theobromine	400	4.1±0.9(2)
Albumin	400	4.2±0.7(2)
Casein	400	3.4±0.8(2)
Citric acid	400	4.0±0.9(3)

a) The per cent absorbed is expressed as the mean ± the range followed by the number of experiments in parentheses.

Table VII. Absorption Rate of CHS-Na from Rat Small Intestine in the Presence of Other Compounds using Isotonic Buffer Solution of pH 7.0

	Concn. in sample soln. (μg./ml.)	Per cent absorbed in 2 hr.[a]
Caffeine	400	13.6±1.8(3)
Theophylline	200	8.9±1.7(3)
Theobromine	400	7.7±1.1(2)
Albumin	400	6.3±0.2(2)
Casein	400	9.1±0.1(2)
Citric acid	400	16.8±4.0(4)

a) The per cent absorbed is expressed as the mean ± the range followed by the number of experiments in parentheses.

which were pH 1.0 in stomach and pH 7.0 in small intestine, were estimated. The results obtained are shown in Tables Ⅵ and Ⅶ. These results showed that caffeine and citric acid accelerated the absorption rate of CHS-Na from the small intestine, but the absorption rate in the stomach was not changed in the presence of those compounds.

Discussion

The relative concentrations of the unionized and the ionized forms of CHS-Na, saccharin, or dulcin, which are given by the Henderson-Hasselbalch equation, are shown in Table Ⅷ. From the results obtained, it is clear that CHS-Na, saccharin, or dulcin exists mostly as unionized forms in the solution of pH 1.0, and that the greatest parts of CHS-Na and saccharin exist as ionized forms in the solutions of pH 3.0, 5.0, or 7.0, while most of dulcin exists as an unionized form in those solutions. Accordingly, it is suggested that CHS-Na is absorbed as an unionized form in the solution of pH 1.0 and as an ionized form in the solutions of pH 3.0, 5.0, or 7.0.

Table Ⅷ. The Relative Concentrations of the Unionized and the Ionized Forms of CHS-Na, Saccharin, and Dulcin

Compound	Unionized form/Ionized form			
	pH 1.0	pH 3.0	pH 5.0	pH 7.0
CHS-Na	8.0	8.0×10^{-2}	8.0×10^{-4}	8.0×10^{-6}
Saccharin	16.0	16.0×10^{-2}	16.0×10^{-4}	16.0×10^{-6}
Dulcin	6.3	6.3×10^{2}	6.3×10^{4}	6.3×10^{6}

These values were calculated by the Henderson-Hasselbalch equation:

for CHS-Na and saccharin $pH=pKa+\log\frac{[\text{ionized form}]}{[\text{unionized form}]}$

for dulcin $pH=pKa+\log\frac{[\text{unionized form}]}{[\text{ionized form}]}$

It was reported that CHS-Na was absorbed sufficiently after oral administration to rabbit*3 or rat.[7] As shown in Tables Ⅱ and Ⅲ, however, CHS-Na was very poorly absorbed from stomach or small intestine of rat *in situ*. As shown in Table Ⅴ, the absorption of CHS-Na was considerably reduced in the pentobarbital-anesthetized animals. Tolazoline, which was rapidly absorbed after oral administration to rat *in vivo*[8] and was very poorly absorbed from stomach or small intestine in rat *in situ*[1,2] was poorly absorbed in rat anesthetized with pentobarbtial. On the other hand, the absorption of sailcylamide was scarcely affected by the use of the anesthetic in rabbit.

It was reported[9] that saccharin appeared in the urine in unchanged form and was excreted completely during 16 to 48 hours after its administration. However, the present experiments showed that the amount of unionized form and the partition coefficient of saccharin were more than those of dulcin in the solution of pH 1.0, but the absorption of saccharin from stomach was less than that of dulcin in rat. Thus the absorption of saccharin *in situ* was seemed to be affected by the use of pentobarbital.

Considering the results above described, it is suggested that the *in situ* absorption of the strong acid and the base (cyclohexylsulfamic acid and tolazoline) are reduced in the treatment of pentobarbital, and that the absorptions of the weak acid and the base (salicylamide and dulcin) are not governed by its effect. Dulcin and salicylamide

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8) L.E. Ellinwood, J.D. Kohli, J.M. Coon: J. Pharmacol. Exp. Therap., **109**, 318 (1953).
9) R.K. Richards, J.D. Taylor, J.L. O'Brien, H. O. Duescher: J. Am. Pharm. Assoc., **40**, 1 (1951).

appear to be absorbed by the simple diffusion mechanism, while the absorption mechanisms of the more polar compounds such as CHS-Na and tolazoline (pKa 10.3)[10] were supposed to be not depend on the lipid theory.[1,2,10,11] Accordingly, such an experiment *in situ* as described above could not exactly inform the features of the absorption of either strong acids or bases.

Furthermore, other compounds such as caffeine, theophylline, theobromine, albumin, casein, and citric acid were used in this experiment to determine the effects on the stomach or the small intestine absorption of CHS-Na. The results of the experiments indicate that caffeine and citric acid accelerate the small intestine absorption of CHS-Na.

Summary

The absorption of CHS-Na, saccharin, or dulcin from the stomach and the small intestine of the anesthetized rat was measured. CHS-Na was very poorly absorbed, while dulcin showed the greatest absorption rate.

The absorpton of CHS-Na was considerably reduced in rat and rabbit anesthetized with pentobarbital. The same results were obtained in the absorption of tolazoline, but were not in that of salicylamide. These results suggest that the decreasing in the absorption of CHS-Na or tolazoline *in situ* depends mainly upon the anesthetic effect of pentobarbital.

And the absorption of CHS-Na was measured in the presence of other compounds. The small intestinal absorption of CHS-Na was accelerated by the addition of caffeine or citric acid to it.

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11) A.M. Hogben, D.J. Tocco, B.B. Brodie, L.S. Schanker: J. Pharmacol. Exp. Therap., **125**, 275 (1959).

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132. Shoji Kojima and Hisashi Ichibagase*1: Studies on Synthetic Sweetening Agents. Ⅷ.*2 Cyclohexylamine, a Metabolite of Sodium Cyclamate.

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Sodium cyclamate (CHS-Na) has widely been used as a noncaloric sweetening agent for various drugs and foods. Some workers[1~3] have already reported on excretion, distribution, and metabolic effect of CHS-Na. Those reports showed that CHS-Na was almost entirely excreted unchanged after administering orally, intravenously, or intraperitoneally, and any metabolites of CHS-Na have not been reported.

*1 Kuhonji, Oemachi, Kumamoto (児島昭次, 一番ヶ瀬 尚).
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